Poor Sleep and Altered Hypothalamic-Pituitary-Adrenocortical and Sympatho-Adrenal-Medullary System Activity in Children

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Context: Neuroendocrine alterations, with well-known links with health, may offer insight into why poor sleep is associated with poor health. Yet, studies testing associations between sleep and neuroendocrine activity in children are scarce.

Objective: The aim of this study was to determine whether actigraphy-based sleep pattern is associated with hypothalamic-pituitary-adrenocortical axis and sympatho-adrenal-medullary system activity in children.

Design and Setting: We conducted a cross-sectional study in a birth cohort in Helsinki, Finland.

Participants: We studied 282 8-yr-old children.

Main Outcome Measures: We measured diurnal salivary cortisol and salivary cortisol and α-amylase (a sympatho-adrenal-medullary system marker) responses to the Trier Social Stress Test for Children (TSST-C).

Results: Children with short ($\leq$7.7 h) *vs.* average sleep duration (7.8–9.3 h) displayed higher cortisol awakening response and nadir ($P < 0.042$). Those with low ($\leq$77.4%) *vs.* average-high sleep efficiency ($>$77.4%) displayed higher diurnal cortisol levels across the entire day ($P < 0.03$), higher cortisol levels after the TSST-C stressor ($P < 0.04$), and higher overall α-amylase levels across the entire TSST-C protocol ($P < 0.05$). The effects were not confounded by factors that may alter sleep or hormonal patterns.

Conclusions: Poor sleep may signal altered neuroendocrine functioning in children. The findings may offer insight into the pathways linking poor sleep with poor health. **(*J Clin Endocrinol Metab* 95: 2254–2261, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-0943 Received May 4, 2009. Accepted February 2, 2010.
First Published Online March 1, 2010

Abbreviations: AUC, Area under the curve; BMI, body mass index; CI, confidence interval; HPAA, hypothalamic-pituitary-adrenocortical axis; MD, mean difference; SAMS, sympatho-adrenal-medullary system; TSST-C, Trier Social Stress Test for Children.

Many children suffer from insufficient sleep quantity and poor sleep quality. Estimates of the prevalence of sleep problems in children vary from 20 to 30%. In nearly half of these children, the problems persist over time (1–5).

Studies in animals and in adult humans have revealed that sleep and the hypothalamic-pituitary-adrenocortical axis (HPAA) show bidirectional effects, with experimental alterations in the HPAA leading to altered sleep and alterations in sleep leading to altered HPAA function (6, 7). Altered HPAA function may, at least in part, be responsible for the effects of poor sleep on health (4, 8) because the HPAA is known to impact health, psychological functioning, and psychopathology (10). However, studies testing the associations between sleep and HPAA functioning in children are scarce. Poor sleep has been assessed with electroencephalogram in 5-yr-old children (11) and with actigraphy in 9-yr-old children (12) and, in both cases, was associated with higher daytime salivary cortisol levels. In 10- to 17-yr-old children, self-reported sleep problems were associated with higher salivary cortisol levels in response to stress (13). However, these studies were limited by their small sample sizes and their reliance on sparse measures of cortisol at a single time point during the day (11, 12) and once after stress (13).

In this study of 282 8-yr-old children, we tested the associations between actigraphy-based sleep pattern with diurnal salivary cortisol pattern and with salivary cortisol responses to a standardized psychosocial stress test, the Trier Social Stress Test for Children (TSST-C) (14, 15). We also tested for associations between sleep pattern and salivary α-amylase responses to stress, a marker of sympatho-adrenal-medullary system (SAMS) activity (16). We hypothesized that short sleep duration and low sleep efficiency would be associated with higher diurnal salivary levels of cortisol and higher salivary cortisol and α-amylase responses to TSST-C stress. Finally, based on findings in adults that showed associations between both short and long sleep duration with poor health (8), we hypothesized that, in addition to short sleep duration, long sleep duration would also be linked with altered levels of salivary cortisol and α-amylase.

Subjects and Methods

Participants

The children were recruited from a random, population-based, urban cohort (initially used to investigate the effects of maternal licorice consumption) (17) comprising initially 1049 infants born between March 1 and November 30, 1998, in Helsinki, Finland, and their mothers. In 2006, children and their parents were invited to participate in a follow-up study with a focus on individual differences in physical and psychological development (18). Financial constraints prohibited inviting the entire initial sample. Thus, those families still living close to or in the greater Helsinki area were invited. Of 413 invited children, 321 (77.7%) agreed to participate (mean = 8.1 yr, SD = 0.3 yr). Of the 321, actigraphy measurements of sleep pattern across at least three nights were obtained from 297 children, and diurnal salivary cortisol and salivary cortisol and α-amylase measurements during stress were obtained from 286, 292, and 288 children, respectively. Altogether, 282 (47.9% male), 281 (47.4% male), and 276 (48.2% male) children had data available for the analyses testing associations of sleep pattern with diurnal cortisol and with cortisol and α-amylase responses to stress, respectively. The analyses excluded children who, according to parental report, had been diagnosed with a developmental delay (n = 3) or Asperger syndrome (n = 1), did not complete the diurnal or stress protocol (n = 17; *e.g.* because the child was ill, family schedule changed, there was dizziness, or saliva was insufficient), or had more than one missing cortisol or α-amylase value (n = 7).

Nonparticipation did not relate to child's gender, birth date, gestational length, weight, length, head circumference, or ponderal index at birth, birth order, mode of delivery, mother's gestational diabetes, gestational hypertension, preeclampsia, age, height, weight, body mass index (BMI), occupational status or blood pressures at delivery (data obtained from birth and medical records), licorice consumption, alcohol consumption, or stress during pregnancy ($P > 0.10$), but was related to more frequent smoking of the mother during pregnancy ($P = 0.02$) (self-reported while still on the maternity ward).

The Ethical Committees of the City of Helsinki Health Department and the Helsinki University Hospital of Children and Adolescents at Helsinki and the Uusimaa Hospital District approved the project. Each parent/guardian and each child gave written informed consent.

Sleep measurement

Sleep pattern was objectively measured using actigraphs (Actiwatch AW4, Cambridge Neurotechnology Ltd., Cambridge, UK). The children wore the actigraph for an average of 7.1 nights (SD = 1.2; range, 3 to 14 nights). The children slept in their own home beds and followed their usual daily schedules pertaining to bed and awakening times. The actigraph yields measurements of sleep duration (hours from sleep onset to awakening) and sleep efficiency (percentage time in minutes asleep while in bed, including time it took to get asleep at bedtime). Parents were asked to keep a sleep log of bed and awakening times. Data were scored with Actiwatch Activity & Sleep Analysis V 5.42 software with medium sensitivity and 1-min epoch duration. The activity data plot was visually inspected to trace significant discrepancies between the sleep log, event markers by the child indicating bed and awakening times, and the activity pattern. Night(s) were excluded from the sleep analysis if: 1) the actigraph was not in use; 2) information on the bedtime was missing; 3) the actigraph data showed the child was already asleep at the reported bedtime; 4) information on awakening time was missing and the activity pattern was not unequivocally interpretable; or 5) the parents reported a significant deviation from normal life due to illness or travel. For 3.6 and 5.1% of the children, the diurnal cortisol protocol was conducted 2 to 150 d before and 2 to 44 d after the sleep assessment, respectively. For 1.1 and 8.7% of the children, the cortisol and α-amylase stress protocols were conducted 27 to 56 d before and 2 to 37 d after

the sleep assessment, respectively. For over 90% of the children, the diurnal and stress protocols were conducted within 1 d of the sleep assessment.

Diurnal cortisol sampling

Parents were shown how to collect salivary samples for determination of cortisol using cotton swabs (Salivette). Salivary samples were obtained during a 1-d period, at awakening (mean = 0753 h; SD = 50 min), 15 and 30 min thereafter, and at 1030 h, 1200 h, 1730 h, and bedtime (mean = 2115 h; SD = 75 min).

Cortisol and α-amylase sampling during the TSST-C stressor

The children were scheduled to arrive in the clinic at 1200 h or at 1400 h and were asked to abstain from eating for 2 h before arrival. After the child and parent/guardian had signed an informed consent, a saliva sample, termed "arrival" hereafter, was obtained. Weight and height of the child were measured, and continuous heart and blood pressure monitoring devices were attached. After the baseline saliva sample was obtained (mean = 36.5; SD = 6.2 min after the arrival sample), the child completed a 5-min baseline recording in a standing position watching a relaxing movie next to the parent before undergoing the TSST-C. The TSST-C elicits reliable HPAA and autonomic responses, which are thought to measure how the child typically responds to stress in his/her normal daily environment. The TSST-C protocol has been described in detail elsewhere (14, 15).

In brief, in the TSST-C, the child was taken to another room without his/her parent/guardian and introduced to a committee of two "judges." A range of toys was presented, and the child was asked to select a favorite and a second favorite. The children were told that they would receive their favorite toy as a reward if they performed tasks extremely well. The two toys were visible on a table between the child and the committee. The beginning of a tape-recorded story was played. The child was asked to plan to complete the story and was told that they would present their story to the committee and that this would be tape-recorded. The child was taken back to the baseline room, without parents present, and prepared the story for 5 min with the support of a research nurse. After this, the child was brought back to the examination room to present the 5-min story. The story was followed by a 5-min mental arithmetic task (counting backward). After completion of the task, the child was rewarded with their favorite toy for an "excellent performance." The child was then led to another quiet room to recover and continue watching the movie started during baseline, with the parent/guardian present.

The TSST-C protocol was performed with the child as standing and the recovery period with the child as sitting, with the exception of a 12-min recovery recording in a standing position, starting 13 min after the TSST-C (15). Salivary samples (Salivette) were obtained at arrival and at baseline, as described above, and immediately and 10, 20, 30, and 45 min after rewarding the child with the favorite toy, which indexed completion of the TSST-C stress protocol.

Biochemical analyses

Salivary cortisol concentrations were determined by use of a competitive solid-phase, time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA; Wallac, Turku, Finland) (19). The assay has a 0.6% cross-reactivity for cortisone. The intraassay coefficient of variation was between 4.0 and 6.7%, and the corresponding interassay coefficients of variation were between 7.1 and 9.0%. Cortisol concentrations were measured in duplicate, and the mean coefficient of variation between duplicate analyses was 5.0%. Salivary α-amylase was determined by use of an enzymatic method (20). The intraassay coefficient of variation was between 3.5 and 5.5%, and the corresponding interassay coefficients of variation were between 5.5 and 7.6%. α-Amylase was also measured in duplicate, with a mean coefficient of variation of 3.7%.

Potential confounders

These included time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C) (21), sex (22), age at testing (22), and BMI (mean = 16.7; SD = 3.3 kg/m^2 calculated from height and weight measured in conjunction with the TSST-C protocol) (23). Furthermore, mothers reported their occupational status, which was categorized according to the classification system of Statistics Finland (low, n = 19 or 6.8%; middle, n = 93 or 33.1%; and upper, n = 169 or 60.1%). While still on the maternity ward, the mothers reported the brand(s) and frequency of their weekly licorice consumption (17). From these, we calculated the quantity of glycyrrhizin in licorice in grams per week (0–249 mg/wk, n = 188 or 66.7%; 250–499 mg/wk, n = 41 or 14.5%; and ≥500 mg/wk, n = 53 or 17.7%).

Statistical analyses

Cortisol and α-amylase concentrations were log-transformed to attain normality. The diurnal hormonal pattern and hormonal response to the TSST-C were first analyzed by linear mixed model analysis (SAS Proc Mixed, SAS Institute Inc., Cary, NC) (24). This analysis is designed for analyzing repeated-measures data with variation arising from both intra- and interindividual differences. One of the main advantages of using mixed model analysis is in the capability to directly measure and specify covariance structure. To test whether the diurnal and the TSST-C hormonal patterns varied according to sleep duration and efficiency, we included an interaction term "sleep duration × sampling time" and "sleep efficiency × sampling time" (both interactions tested separately) into the regression equation, followed by the main effects.

Second, whether or not more conventional indicators of the diurnal and the TSST-C hormonal patterns varied according to sleep duration and efficiency was determined by using multiple linear regression analyses. Diurnal variables were cortisol peak value after awakening (peak of values 15 and 30 min after awakening), cortisol awakening response (peak value after awakening minus value at awakening), awakening time-weighted area under the curve (AUC of 0, 15, and 30 min after awakening, calculated as the AUC above zero under trapezoidal rule), awakening AUC increment (AUC minus awakening value), and nadir (minimum of diurnal values). TSST-C stressor variables were baseline, peak value after stress, increment (peak value after stress minus baseline value), time-weighted AUC (calculated as the AUC above zero under trapezoidal rule), and AUC increment (AUC minus baseline value).

All analyses were computed with sleep duration and efficiency, first as continuous variables, and second as categorical. Following the lead of others (25) and our previous analyses (26, 27), sleep duration was categorized into three groups with short (≤7.7 h, ≤10th percentile) and long (≥9.4 h, ≥90th percentile) sleepers contrasted with average sleepers as the referent (7.8–9.3 h, 10th-90th percentiles), whereas sleep efficiency (≤77.4%,

≤10th percentile *vs.* others) was dichotomized. All analyses were also computed with and without confounders. Because the unadjusted and fully adjusted models resulted in essentially similar results, we present the fully adjusted associations only.

Furthermore, although more than 90% of the children underwent the sleep assessment within 1 d of the diurnal and stress protocols, this was not the case for all the children. Therefore, we repeated all the analyses by including the gap in time between the protocols as an additional covariate. Finally, because associations with cortisol (22) and α-amylase (28) may vary according to sex, we tested whether sex moderated any of the associations. In no instance was there a significant sex-interaction term ($P > 0.07$) (data not shown). For this reason, we report the results in both sexes combined.

Results

In comparison to girls, boys had a shorter mean sleep duration (8.5 *vs.* 8.3 h; $P = 0.002$) and a lower mean sleep efficiency (85.1 *vs.* 83.5%; $P = 0.02$). The sleep pattern did not vary significantly according to age and BMI of the child or occupational status of the mother (all P values >0.08). Sleep duration correlated significantly with sleep efficiency ($r = 0.80$; $P < 0.001$).

The later the children awoke, the lower were their diurnal salivary cortisol levels upon awakening (−12.0%/h; $P = 0.01$). After awakening, each child's level of salivary cortisol decreased substantially until bedtime (−14.8%/h; $P < 0.0001$). Salivary cortisol and α-amylase at arrival to the clinic or at baseline of the TSST-C were not significantly associated with time of day ($P > 0.18$), but increased by 3.7% ($P = 0.005$) and 2.6% ($P = 0.005$) per each 10-min increase in time after stress, respectively. In 44.8 and 53.7% of the children, salivary cortisol and α-amylase had peaked 20 min after the TSST-C, respectively. Boys (relative to girls) and older (relative to younger) children displayed lower cortisol levels after the TSST-C ($P < 0.05$). There were no other significant associations between sex, age, BMI, or mother's occupational status and hormonal parameters.

Sleep and diurnal salivary cortisol

Continuous measures of sleep duration were not associated with diurnal cortisol pattern or with conventional indices of diurnal HPAA activity ($P > 0.14$). When sleep duration was categorized, short sleepers showed a trend toward higher overall level of diurnal salivary cortisol [mean difference (MD) = 19.6%; 95% confidence interval (CI), −1.14 to 40.4; $P = 0.059$] and toward smaller decrease in diurnal salivary cortisol across the day, compared with average sleepers (sleep duration × sampling time; $P = 0.064$) (Fig. 1). Analyses of the conventional HPAA indices showed that short sleepers displayed a higher cortisol awakening response (Fig. 2A) and nadir (Fig. 2B) than average sleepers. Long and average sleepers did not differ from each other ($P > 0.10$) (Figs. 1 and 2).

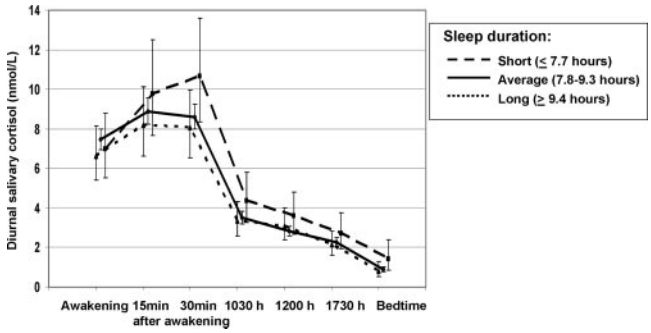

FIG. 1. Diurnal salivary cortisol values in children with short (≤7.7 h), average (7.8–9.3 h), and long (≥9.4 h) sleep duration. Values are geometric means, and error bars are 95% CIs adjusted for time at awakening, sex, age, and BMI, mother's occupational status, and mother's licorice use during pregnancy. Decrease in salivary cortisol per hour, −12.6%, −14.3%, and −15.3% for short, average, and long sleepers, respectively (all P values <0.001).

The lower the children's sleep efficiency was, the higher the overall level of salivary cortisol (11.0% increase per 10% decrease in continuous sleep efficiency; 95% CI, 1.0 to 22.0, $P = 0.038$; MD = 33.2% between the low *vs.* average-high sleep efficiency groups; 95% CI, 13.4 to 53.0, $P = 0.001$), and the smaller the decrease in diurnal salivary cortisol across the day (continuous sleep efficiency × sampling time, $P = 0.009$; dichotomous sleep efficiency × sampling time, $P = 0.018$) (Fig. 3A). Analyses of the conventional HPAA indices showed that children with low *vs.* average-high sleep efficiency displayed higher cortisol upon and after awakening, higher awakening AUC, and higher nadir (Table 1).

Sleep and salivary cortisol responses to TSST-C stressor

Sleep duration, either as continuous or categorical, and sleep efficiency as continuous was unrelated to cortisol responses to TSST-C. As shown in Fig. 3B, children with low *vs.* average-high sleep efficiency displayed, however, a higher level of cortisol after the TSST-C (sleep efficiency × sampling time, $P = 0.027$). Furthermore, they had a higher peak level, increment, AUC, and AUC increment of salivary cortisol in response to TSST-C (Table 1).

Sleep and salivary α-amylase responses to TSST-C stressor

α-Amylase responses to TSST-C did not vary significantly according to continuous or dichotomous sleep duration or efficiency ($P > 0.10$). However, the lower the children's sleep efficiency, the higher the overall level of salivary α-amylase across the entire TSST-C protocol (1.5% increase per each 10% decrease in efficiency; 95% CI, 0.0 to 3.0, $P = 0.050$; MD = 32.9% between low *vs.*

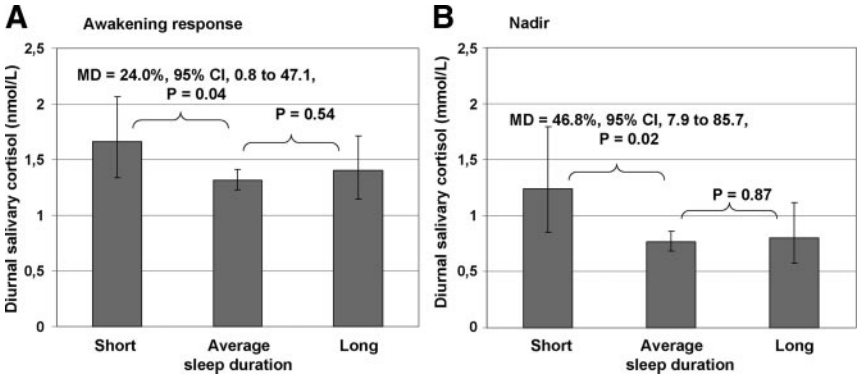

FIG. 2. Diurnal salivary cortisol awakening response (peak value of 15 and 30 min after awakening minus value at awakening) (A), and nadir (minimum of diurnal values) (B) according to short (≤7.7 h), average (7.8–9.3 h), and long (≥9.4 h) sleep duration. Values are geometric means, and error bars are 95% CIs adjusted for time at awakening, sex, age, and BMI, mother's occupational status, and mother's licorice use during pregnancy.

average-high sleep efficiency groups; 95% CI, 1.7 to 64.1, $P = 0.036$). Children with low *vs.* average-high sleep efficiency displayed higher peak level of salivary α-amylase in response to TSST-C (Table 1).

When the gap in time between the sleep assessment and diurnal and stress protocols was included in the analyses as an additional covariate, the results changed little (all P values <0.05), except for one. The difference in diurnal salivary cortisol awakening AUC increment between short and average sleepers became significant, with short sleepers displaying a higher increment (MD = 22.4%; 95% CI, 4.3 to 40.5; $P = 0.016$).

Finally, according to parent reports, a number of children had been diagnosed (by a physician) with medical disorders that may interfere with sleep, including asthma (n = 16), atopic eczema (n = 37), allergic rhinitis (n = 24), and dysphasia (n = 4) and dyslexia (n = 2). Similarly, TSST-C may induce a higher stress response in children with dysphasia/dyslexia. Therefore, we repeated all the analyses by adding these parameters as covariates. The results that were statistically significant remained so after the adjustments ($P < 0.038$), except for one. The difference in salivary cortisol AUC increment in response to TSST-C between children with low and average-high sleep efficiency became nonsignificant ($P = 0.054$).

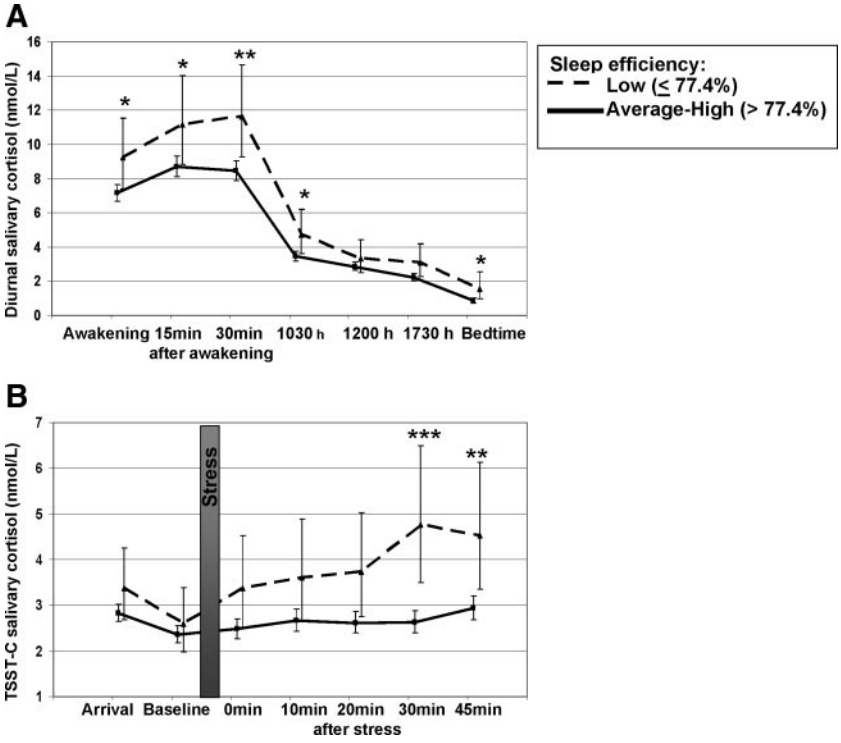

FIG. 3. Diurnal salivary cortisol values (A) and salivary cortisol responses to TSST-C (B) in children with low (≤77.4%) and average-high (>77.4%) sleep efficiency. Values are geometric means, and error bars are 95% CIs adjusted for time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C), sex, age, and BMI, mother's occupational status, and mother's licorice use during pregnancy. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Decrease in diurnal salivary cortisol levels, per hour, −11.8 and −15.4%, and increase in salivary cortisol levels after stress, per 10 min, 12.0 and 4.6% for children with low and average-high sleep efficiency (all P values <0.002).

Discussion

To our knowledge, this is the first study to show that poor sleep in children is associated with altered diurnal and stress-induced HPAA function. Children who were at the low end of the distribution of sleep duration differed from average sleepers on two indices of diurnal salivary cortisol. Children with short, compared with average sleep, displayed higher salivary cortisol response to awakening and higher salivary cortisol nadir. Sleep efficiency, however, did show a more consistent pattern of associations with diurnal and stress-induced HPAA. The lower the children's sleep efficiency was, the higher the children's level of salivary cortisol across the entire day. Children with low sleep efficiency also displayed higher levels of salivary cortisol after the TSST-C stressor, but the higher levels characterized only those children who were at the low end of the distribution in sleep efficiency. These associations were not confounded by differences in time of day, sex, age, BMI, maternal occupational status, maternal licorice consumption during pregnancy, gap in time between the sleep

TABLE 1. Geometric means of diurnal salivary cortisol, and of salivary cortisol and salivary α-amylase during the TSST-C according to sleep efficiency

	Sleep efficiency, mean (95% CI)			
	Low (≤77.4%)	Average-high (>77.4%)	MD in % (95% CI)	*P*
Diurnal salivary cortisol (nmol/liter)				
Upon awakening	9.3 (7.5 to 11.5)	7.2 (6.7 to 7.7)	25.8 (2.9 to 48.7)	0.027
Peak after awakening	13.1 (10.5 to 16.3)	9.7 (9.1 to 10.4)	30.5 (7.5 to 53.5)	0.010
Awakening response	1.4 (1.1 to 1.7)	1.3 (1.3 to 1.4)	4.7 (−17.9 to 27.3)	0.68
Awakening AUC	10.7 (8.8 to 13.1)	8.2 (7.7 to 8.7)	27.5 (6.6 to 48.4)	0.010
Awakening AUC increment	1.2 (1.0 to 1.4)	1.2 (1.1 to 1.2)	2.1 (−14.5 to 18.6)	0.81
Nadir	1.3 (0.9 to 1.8)	0.8 (0.7 to 0.9)	51.5 (14.0 to 88.9)	0.007
Salivary cortisol during the TSST-C stressor (nmol/liter)				
Baseline	2.5 (1.9 to 3.3)	2.3 (2.2 to 2.5)	6.7 (−21.4 to 34.7)	0.64
Peak after stress	6.1 (4.5 to 8.3)	3.8 (3.5 to 4.2)	46.2 (14.0 to 78.5)	0.005
Increment	2.4 (1.8 to 3.2)	1.6 (1.5 to 1.8)	39.6 (10.2 to 68.9)	0.009
AUC	3.5 (2.8 to 4.5)	2.6 (2.4 to 2.8)	30.3 (4.3 to 56.4)	0.022
AUC increment	1.4 (1.1 to 1.7)	1.1 (1.0 to 1.2)	23.7 (1.5 to 45.8)	0.037
Salivary α-amylase during the TSST-C stressor (U/ml)				
Baseline	66.2 (47.5 to 92.5)	47.3 (42.7 to 52.4)	33.4 (−1.7 to 68.4)	0.06
Peak after stress	88.0 (64.0 to 120.9)	60.8 (55.2 to 67.0)	36.7 (3.3 to 70.1)	0.03
Increment	1.3 (1.2 to 1.5)	1.3 (1.2 to 1.3)	3.3 (−10.6 to 17.2)	0.64
AUC	62.0 (44.7 to 85.9)	45.3 (40.9 to 50.0)	31.1 (−3.2 to 65.4)	0.08
AUC increment	0.9 (0.8 to 1.0)	1.0 (0.9 to 1.0)	−2.3 (−13.3 to 8.8)	0.69

Diurnal variables: peak value after awakening, peak of values 15 and 30 min after awakening; cortisol awakening response, peak value after awakening minus value upon awakening; AUC, awakening time-weighted AUC of 0, 15, and 30 min after awakening, calculated as the AUC above zero under trapezoidal rule; awakening AUC increment, AUC minus awakening value; nadir, minimum of diurnal values. Stress response variables: peak after stress, peak of 0, 10, 20, 30, and 45 min after stress; increment, peak after stress minus baseline value; AUC, time-weighted AUC of baseline, 0, 10, 20, 30, 45 min after stress calculated as the AUC above zero under trapezoidal rule; AUC increment, AUC minus baseline value. All values are adjusted for time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C), sex, age, and BMI, mother's occupational status, and mother's licorice use during pregnancy.

assessment and the diurnal and stress protocols, and impairments of the child that may alter sleep and hormonal patterns. Our study thus extends the scant prior evidence in children (11–13) by showing that in a population of generally healthy 8-yr-olds from families where the majority of mothers were well-educated, poor sleep may signal altered neuroendocrine functioning.

The cross-sectional study design does not allow inferences of causality. However, the existing experimental evidence in adult humans suggests that sleep and the HPAA activity may be causally and reciprocally related. Experimental sleep deprivation in adults induces HPAA activation by increasing evening cortisol levels in particular (29, 30). There is also evidence in adults that arousals induced during sleep and subsequent nocturnal awakenings stimulate an increase in cortisol release (31). Experimental administration of glucocorticoids (32) or CRH (33), in turn, negatively impact on sleep by promoting light sleep and nocturnal awakenings in adults. Indeed, preliminary clinical evidence in adults suggests that interventions targeted at suppressing CRH activity may be effective in treating insomnia (34–36). Furthermore, higher levels of cortisol in the evening, while still awake, have been associated with more frequent subsequent nocturnal awakenings in adults (37). Because activation of the HPAA promotes insulin resistance, accumulation of visceral fat, and hypertension and is among the most consistently demonstrated biological abnormalities related to mood disorders (10), our findings, if causal, may offer insight into the mechanisms underlying the link between poor sleep, regardless of age, and poor health (8, 9, 37, 38). Indeed, there is consistent evidence that children who are short sleepers are obese and consequently are at risk for the many adverse biological and psychological effects of obesity (37). Furthermore, even when statistical analyses control for BMI, short sleep duration and low sleep efficiency are associated with prehypertensive status among adolescents (38).

Salivary α-amylase, an enzyme that is responsible for carbohydrate digestion, is released in response to stress under sympathetic neurohormonal stimulation and is, therefore, considered to be a potential marker of the activity of the SAMS (16). We did not find associations between poor sleep and α-amylase response to stress but found, instead, that in children with low, compared with average-high sleep efficiency, the peak level of α-amylase after stress and the overall α-amylase level across the entire stress protocol were higher. This perhaps indicates chronic SAMS activation in addition to HPAA activation, rein-

forcing the neuroendocrine implications of poorer sleep. There are no previous studies linking sleep pattern with α-amylase, and relatively little, in general, is known about its health consequences. Thus, whether salivary α-amylase may participate in the chain linking sleep with health is not clear.

Apart from a cross-sectional study design, other limitations of our study relate to generalizability of the findings. Sleep (39) and hormonal patterns (22) change with age and pubertal maturation. Thus, our findings cannot be generalized to younger or older age groups. Also, more than half of the sample had mothers with a higher occupational status (*e.g.* doctors, teachers). Although we did not observe associations between maternal occupational status and sleep and hormonal patterns, the findings may not generalize to less affluent populations. Furthermore, the children's salivary cortisol curve in response to the TSST-C stressor differed from that usually seen in adult populations and in some other populations of healthy children (15). This was true, despite evidence that the salivary cortisol level at the time when the TSST-C task was introduced was comparable to the level interpolated from the diurnal salivary cortisol curve (data not shown). Recently, however, it has been recognized that many healthy children may be relatively cortisol hyporesponsive to a variety of stressors, including the TSST-C (40). Although our data do demonstrate that in a majority of the children, salivary cortisol levels increased in response to the TSST-C, with some children showing a "late" response, further studies are clearly warranted to understand variations in stress-induced salivary cortisol patterns in different populations of children. A further limitation relates to the gap in time between the sleep assessment with the diurnal and stress protocols: over 90% of the children underwent the diurnal and stress protocols within 1 d of the sleep assessment, whereas the rest underwent the diurnal and the stress protocols before or after. When gap in time between the protocols was introduced as an additional covariate, our results changed only slightly. We used actigraphy instead of polysomnography for measuring sleep pattern, although the agreement rates are high (85 to 89%) (41). In addition, although polysomnography is likely to be more accurate in measuring total sleep time among young children (9), actigraphy has the benefit of allowing examination of sleep patterns over many consecutive nights in a natural home setting (41). Finally, whereas sleep and HPAA may be causally related, a possibility remains that the associations are determined by common underlying factors, such as genetic markers for sleep-wake cycles.

In summary, our study shows that short sleep duration is associated with higher levels of salivary cortisol in response to awakening and toward bedtime, and that low sleep efficiency is associated with higher diurnal levels of salivary cortisol across the entire day and after stress. For children with low sleep efficiency, salivary α-amylase levels are also higher across the entire stress protocol. The findings may offer insight into why poor sleep is associated with poor health.

Acknowledgments

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This work was supported by grants from the Juho Vainio Foundation, the John D. and Catherine T. MacArthur Foundation, the European Science Foundation (EuroSTRESS), and the Academy of Finland.

Disclosure Summary: The authors disclose no conflict of interest.

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